Studies on oral immunotherapy-induced immune changes at the local GI tissue through transcriptomics

Wenning Zhang1, Gopal Krishna Ramadas Dhondalay1, Ramona Hoh2, Dana Tupa1, Bryan J. Bunning1, Nielsen Q. Fernandez-Becker1, Neeraja Kambham2, Scott Boyd1,2, Stephen Galli1,2, Sandra Andorf1, Monali Manohar1, R. Sharon Chinthrajah1 & Kari Nadeau1
1. Sean N. Parker Center for Allergy & Asthma Research, Stanford University, Stanford, CA
2. Department of Pathology, Stanford University, Stanford, CA

BACKGROUND
The findings from our phase 2, randomized, placebo-controlled peanut oral immunotherapy (OIT) trial (NCT02103270: POISED study) showed that peanut OIT could desensitize individuals with peanut allergy to 4000 mg peanut protein (Chinthrajah, RS, Lancet 2019, vol 394, P1437-1449).

Peanut Oral Immunotherapy: Safety, Efficacy and Discovery

While peripheral blood is the most accessible tissue to study the immune mechanisms behind food allergy and OIT, examining such mechanisms in the GI-resident immune cells will provide a far better, clinically relevant insight. We thus aimed to investigate immune changes in the biopsied GI tissue from a subset of participants from POISED study before and after peanut OIT using RNA-Seq.

METHODS

Bulk Tissue RNA-seq | RNA extraction, generation of cDNA libraries, sequencing, and data analysis

RNA from a set of site-specific biopsies was extracted with Qiagen RNaseq mini spin column and subsequently pooled to generate cDNA libraries with TakaraBio SMARTer kits and sequence on Illumina HiSeq4000. The raw files were quality-checked with FastQC, aligned to human genome (GRCh38) with STAR, and quantified for gene-level counts using RSEM method. DESeq2 was used for differential expression analysis (log2FoldChange > 2; FDR < 0.01). The Pathway enrichment analysis was done using KEGG package.

Sorted Cell RNA-seq | Sample Processing, Cell Sorting

A set of five site-specific biopsies was pooled, homogenized and digested with Collagenase IV. The resulting cell suspension was stained with the following fluorescent mAbs: gamma delta TCR-PE, CD4-APCCy7, CD3-BV605, and Live/Dead-Aqua dye. Gamma delta T cells were gated as shown below and bulk sorted in 100 μl PBS, pelleted, and kept frozen and kept frozen at -80°C until RNA extraction.

RESULTS

Bulk Tissue RNA-seq | Differential Expression Analysis

Active arm | Week 0 Vs Week 104, N = 7

Transcriptomic analysis from participants undergoing active peanut OIT compared to their self-matched baseline (N=3) showed 1716 differentially expressed genes on comparison of pre- vs. post-OIT in the active arm in gd T cells.

Sorted Cell RNA-seq | Differential Expression Analysis

Active arm | Week 0 Vs Week 104, N = 3

Transcriptomic analysis from participants undergoing active peanut OIT compared to their self-matched baseline (N=3) showed 34 differentially expressed genes on comparison of pre- vs. post-OIT (i.e. week 0 vs week 104) in the active arm.

Ongoing Work

• Further analysis of RNA-seq results
• TCR-seq and Multiplex Ion Beam Imaging(MIBI)
• Confocal visualization of immune markers and tight junction markers on FFPE duodenum sections
• Secretory IgA level testing in stool samples

Funding

This work was supported through the Sean N Parker Center for Allergy and Asthma Research at Stanford University, NIAID AADCRC U19AI104209.